

6 Crohn's disease

Anti-Tumor Necrosis Factor Treatment Restores the Gut Barrier in Crohn's Disease

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OBJECTIVES: A primary defect of the tight junctions and, hence, increased intestinal epithelial permeability has been proposed as a basic pathogenic event in Crohn's disease. Challenge of the mucosal immune system by the commensal gut flora would then result in chronic inflammation. Alternatively, increased permeability could be the result of inflammation. Our aim was to study intestinal permeability in refractory Crohn's disease before and after treatment with monoclonal chimeric antibodies directed against tumor necrosis factor (TNF) to investigate whether the abnormal permeability persists after control of inflammation.

METHODS: Twenty-three patients with active Crohn's disease were evaluated before and 4 wk after a single infusion of 5 mg/kg infliximab. Intestinal permeability was studied by measurement of urinary excretion of ^{51}Cr -EDTA after oral intake.

RESULTS: The increased permeation of ^{51}Cr -EDTA through the small intestine (1.63% interquartile range [IQR] 1.06–2.07) and the overall permeation (3.27% IQR 2.40–4.38) before therapy decreased significantly after infliximab infusion to values (1.04% IQR 0.74–1.54 and 2.42% IQR 2.03–2.80, respectively) in the range of those found in normal volunteers (1.12% IQR 0.85–1.58 and 2.28% IQR 1.88–2.86, respectively).

CONCLUSION: Inhibiting the proinflammatory cytokine tumor necrosis factor dramatically reduces gut inflammation and largely restores the gut barrier in Crohn's disease. Our data confirm the central role of TNF in gut barrier modulation in inflammatory conditions *in vivo*. (Am J Gastroenterol 2002;97:2000–2004. © 2002 by Am. Coll. of Gastroenterology)

INTRODUCTION

In 1972 Shorter *et al.* (1) raised the hypothesis that the basic defect in Crohn's disease (CD) might be an abnormal epithelial gut barrier consisting of a single layer of columnar epithelial cells joined together by tight junctions (2). Increased passage of antigens through the intestinal mucosa then would inadequately activate the immune system, lead-

ing to chronic gut inflammation. The concept of a "permeable" epithelial intestinal barrier in Crohn's disease has gained much support since. Moreover, ultrastructural studies using freeze fracture have shown abnormalities of the tight junctions even in apparently unaffected bowel segments of patients with Crohn's disease (3). Experimental studies in rats have shown that tight junctional permeation of ^{51}Cr -EDTA (^{51}Cr -labeled ethylenediaminetetraacetate) correlates well with the uptake of macromolecules in inflamed segments of small bowel (4).

An important step in permeability research in Crohn's disease was the development of noninvasive methods for measuring permeability *in vivo*. Intestinal barrier dysfunction in inflammatory bowel disease (IBD), as evidenced by increased ^{51}Cr -EDTA permeation, was first demonstrated by Bjarnason *et al.* in 1983 (5). Teahon *et al.* further validated this method (6). These authors found a good relationship between the site of increased ^{51}Cr -EDTA permeation and the location of the disease in the intestine. An abnormal overall gut permeability has since been documented in a large proportion of the patients with CD in many studies (7–12). Increased permeation of macromolecules (13) as well as of ^{51}Cr -EDTA (14) has also been demonstrated in macroscopically noninvolved bowel in patients with Crohn's disease.

In 1986, Hollander *et al.* (15) found increased intestinal permeability for PEG-400 in a proportion of first degree relatives of Crohn's disease patients. This finding has been confirmed for all other probes, and it is now well established that 10–25% of first degree relatives of Crohn's disease patients also have a leaky gut (16–18). These findings point toward a primary defect that might be genetically inherited.

However, several studies showed that intestinal permeability is restored when remission is obtained in many patients with active Crohn's disease by treatment with an elemental diet (19–21).

Prednisolone therapy also may decrease permeability in active Crohn's disease and ulcerative colitis in children and adolescent patients (22). These data suggest a close relationship between inflammatory activity and gut permeability. Moreover, some studies suggest that a persisting in-

Table 1. Crohn's Disease Patient Data

Age at onset (yr)	25 (range 12-67)
Disease location	No. of patients
Ileitis	6
Ileocolitis	11
Colitis	6
Previous surgery	7
Therapy	
Glucocorticosteroids	13
Immunosuppressives (azathioprine)	5
Glucocorticosteroids & immunosuppressives	3
Mesalazine/salazopyrine	11/1

creased permeability is a risk factor for relapse in Crohn's disease (23).

The antigenic trigger most likely involved in activation of the gut immune system is the commensal gut flora. This flora is critical for the development of chronic inflammation in most models of IBD (24). Also in Crohn's disease in human patients, the luminal flora, their products, and dietary antigens play a key role in the initiation of early lesions, in the modulation of the activity of the disease, and in the perpetuation of the inflammation (25-28). The question thus remains whether patients with CD have a leaky gut to start with, or whether the increased permeability is the consequence of inflammation.

Our hypothesis was that reducing the inflammation with monoclonal antibodies directed against tumor necrosis factor (TNF) might restore the barrier dysfunction associated with inflammation or expose a pre-existing underlying gut barrier defect.

The present study shows that chimeric monoclonal antibodies directed against TNF restore the gut barrier, and thus confirms that TNF plays a key role in gut barrier function *in*

vivo in inflammatory conditions. This study also shows that inflammation and gut permeability are closely interrelated.

MATERIALS AND METHODS

Intestinal permeability for ^{51}Cr -EDTA (Amersham International, Amersham, UK) was measured in 23 patients (14 female and nine male) with a median age of 33 yr (range 19-72 yr) with refractory active Crohn's disease, 2 days before and 4 wk after the first infusion of infliximab (Remicade, Centocor, Malvern, PA) 5 mg/kg. The patient data are shown in Table 1. Patients continued their treatment before and after the administration of anti-TNF- α .

Thirty-one healthy volunteers (18 female and 13 male) with a median age of 23 yr (range 21-50) performed the same test.

Clinical Scores

The Crohn's Disease Activity Index (CDAI) (29) was measured in the week preceding the permeability test and C-reactive protein (CRP) in serum was assessed on the day of the test. CDAI and CRP are expressed as median (interquartile range [IQR]).

Permeability Test

After an overnight fast the subjects drank 160 ml of Nutridrink (390 mOsm/L) (Nutricia, Bornem, Belgium) with 50 μCi ^{51}Cr -EDTA and rinsed the glass with 90 ml of water (30). Eating or drinking was not allowed for the next 2 h. Urine was collected for 24 h fractionated from 0-6 h and 6-24 h; the former measures permeation mainly in the small bowel, whereas the 0-24 h value is representative of whole gut permeation. Alcohol and nonsteroidal anti-inflammatory drug intake was prohibited in the 24 h and 1 wk, respec-

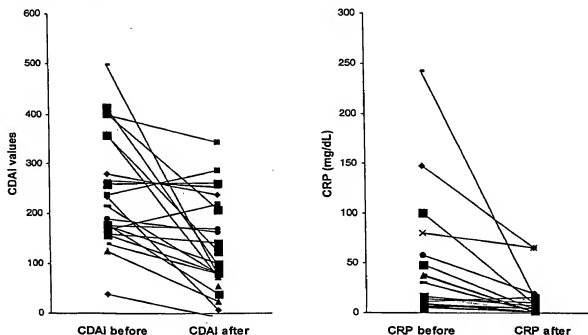


Figure 1. Individual values of CDAI and CRP before and after treatment.

Table 2. Median Permeability Values of Control Subjects and Crohn's Disease Patients

	% Dose Recovery, median (IQR)	
	0-6 h	0-24 h
Control values	1.12 (0.85-1.58)	2.28 (1.88-2.86)
Before anti-TNF	1.63 (1.06-2.07)*	3.27 (2.40-4.38)*
After anti-TNF	1.04 (0.74-1.54)†	2.42 (2.03-2.80)†

*Crohn's disease-patients compared to control subjects; $p = 0.05$.†Before and after infliximab; $p = 0.05$.

tively, preceding and during the test. Volumes of urine were recorded and 1-ml aliquots were counted for radioactivity by a β liquid scintillation counter (Packard, model 2100, Downers Grove, IL) within 48 h after sampling. Results were expressed as the percent urinary excretion of the orally administered dose of ^{51}Cr -EDTA. Background radiation, defined as radiation measured in the absence of ^{51}Cr -EDTA, was negligible.

Statistical Analysis

Logarithmic transformation of the permeability values yielded normal distributions in the Crohn's disease and control groups. Results were considered statistically significant when the two-sided probability was less than 0.05. A paired t test was performed in the Crohn's disease group, along with an unpaired t test to compare the CD and control group permeability values. The CDAI values were not normally distributed and were compared with the Wilcoxon matched pairs test.

The CRP values had a normal distribution and were compared with a paired t test. Fisher exact test was used to evaluate the relation between permeability and treatment response. All tests were performed with the Statistica program on PC (StatSoft, Tulsa, OK).

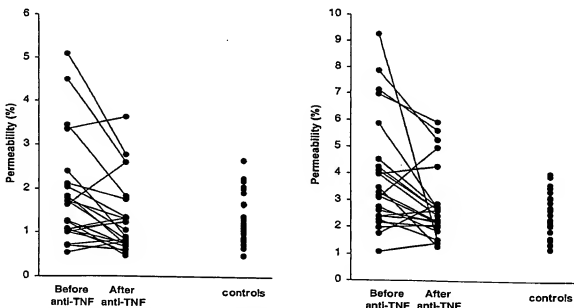
Study Approval

The study was approved by the Ethical Committee of the University and all patients gave their written informed consent for the study.

RESULTS

The CDAI decreased significantly ($p < .01$) from 225 (170-264) to 99 (74-158) and CRP from 14.9 (9.1-43) to 3.7 (1-11.6) mg/L under anti-TNF treatment. The individual values are shown in Figure 1. Urinary volumes before and after infliximab treatment did not differ significantly. Intestinal permeation values for ^{51}Cr -EDTA were independent of the urinary volumes. Table 2 shows the results of intestinal permeabilities for ^{51}Cr -EDTA expressed as median percentage and interquartile range (p25-p75) for the patients before and after therapy and for normal controls. The small bowel and whole gut permeation of ^{51}Cr -EDTA (0-6 h and 0-24 h) in CD patients before treatment was significantly higher than in control subjects. The small intestinal and whole gut permeability in CD patients decreased significantly to normal mean values after treatment. The individual permeation values are shown in Figure 2. In the patients with very elevated permeation before therapy anti-TNF decreased permeation but did not completely bring it back to values comparable to those seen in normal volunteers. In contrast it is striking that, in most patients with permeation values that were within normal limits before therapy, anti-TNF still decreased permeation, suggesting that the absolute permeability values may not be so important.

The decrease in whole gut permeation for ^{51}Cr -EDTA paralleled clinical improvement and the improvement of acute phase reactants in 77% and 78% of the patients, respectively. Intestinal permeability did not correlate signif-

**Figure 2.** Individual small bowel (left) and whole gut permeability (right) values in CD patients and controls.

icantly with response outcome, probably because of the limited number of patients included.

DISCUSSION

The dramatic clinical effect of anti-TNF treatment in refractory Crohn's disease has clearly been shown by the pilot trial by Van Dullemen *et al.* (31) and the pivotal placebo controlled trial by Targan *et al.* (32). In patients responding to this anticytokine therapy, rapid and thorough endoscopic and histological healing of the bowel has been demonstrated (33). Moreover, immunohistochemical studies have shown important downregulation of proinflammatory cytokines and adhesion molecules in Crohn's disease mucosa (34).

The present study shows for the first time *in vivo* that neutralization of the proinflammatory cytokine TNF not only suppresses bowel inflammation but also largely restores the gut barrier in Crohn's disease, offering evidence for the hypothesis that the alterations of the gut barrier in CD are induced by proinflammatory cytokines.

It is clear from this study that suppression of inflammation and barrier restoration are both induced by the same cytokine antibody. This implies that inflammation and barrier function are closely interrelated. The hypothesis of a primary role of hyperresponsiveness of the gut barrier to gut flora or other exogenous factors in the initiation or relapse of the intestinal inflammation still may stand. Treatments such as an elemental diet (19–21) or prednisolone (22) have already shown to induce clinical remission and a decrease in gut permeability in active Crohn's disease. The precise importance of the present study lies in the fact that precise targeting by human chimeric monoclonal antibodies, shutting off one cytokine, is able to alter an essential property such as gut barrier function *in vivo*.

In the healthy normal bowel, "controlled inflammation" regulates contact with the potentially harmful luminal environment (35). In Crohn's disease there is an increased influx of inflammatory effector cells and upregulation of proinflammatory cytokines and other mediators of inflammation, and this delicate balance is disturbed. Cytokines such as interleukin-4 (36, 37), interferon- γ (IFN- γ) (38, 39) and TNF (40, 41) increase epithelial permeability through modulation of tight junction structure in intact monolayers. Serosal TNF decreased transepithelial resistance in the colonic epithelial cell line HT-29/B6 accompanied by a decrease in the number of tight junction strands, and adding IFN- γ to TNF further synergized in impairing the intestinal barrier (40). Zareie *et al.* demonstrated the capability of macrophages activated *in vitro* by *Salmonella minnesota* lipopolysaccharide and an *E. coli*-derived tripeptide, of mediating epithelial barrier function in T84 monolayers (41). The macrophage-derived TNF formed the key cytokine in these epithelial changes by means of an autocrine mechanism, as was shown by addition of a neutralizing antibody against TNF (41). TNF thus plays a critical role in modulating barrier function in IBD, through either direct or in-

direct action on the intestinal epithelium *in vitro*. The present *in vivo* study confirms that TNF exerts a significant effect on the intestinal barrier, and thus underlines the key role of TNF in the immunomodulation of gut permeability in inflammatory conditions.

In conclusion, we have shown that anti-TNF treatment largely restores gut barrier function in Crohn's disease. We hypothesize that the first event in the initiation of CD is aberrant activation of macrophages in the mucosa leading to increased production of proinflammatory cytokines responsible for disrupting the gut barrier. Permeation of gut flora components then further upregulates and perpetuates inflammation, leading to full-blown Crohn's disease.

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